Review

Statins: candidates for promoting bone formation via BMP-2

Masahiko Mori1,2, Tetsunari Nishikawa1, Kazuya Masuno1, Tomoharu Okamura1, Akio Tanaka1, Michio Shikimori2

1Department of Oral Pathology, Osaka Dental University, Hirakata, Japan
2Department of Oral Maxillofacial Surgery, Asahi University, School of Dentistry, Mizuho, Japan

Abstract: Statins are a class of drugs widely used in the treatment of cardiovascular diseases to reduce hypercholesterolemia, and involve an increased prevalence of osteoporosis. Several studies suggested that statin mediates stimulation of bone remodeling. One of the statin family members was first isolated from Penicillin citrium (mevastatin), and another later from Aspergillus terreus (lovastatin). Roles of statins are mediated by competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, such as inhibition of the mevalonate pathway. Statins have a potential anti-inflammatory effect (IL-6), which may be related to promotion of bone growth and maintaining bone health. Statins in local use and general administration enhance osteoblastic differentiation and production of bone matrix proteins via bone morphogenetic protein 2 (BMP-2) signaling pathways. Clinical applications of statins have increasingly been reported for bone fractures and implant dentistry. The present review introduces biophysiological functions of the statin family in normal remodeling of bone tissues demonstrated by both in vivo and in vitro studies, and in promoting bone growth caused by osteoprotegerin production by statins with BMP-2.


Key words: BMP-2, bone, HMG-CoA, osteogenesis, statin

Correspondence: Tetsunari Nishikawa, Department of Oral Pathology, Osaka Dental University, 8-1, Kuzuha-Hanazono-cho, Hirakata 573-1121, Japan
Phone: +81-72-864-3057, Fax: +81-72-864-3157, E-mail: tetsu-n@cc.osaka-dent.ac.jp

Introduction

Statins are a group of drugs which competitively inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and include such natural products as lovastatin, as well as chemically-modified simvastatin and pravastatin. The statin family has been widely used for the treatment of hypercholesterolemia, although statins were initially reported to stimulate rodent bone formation in association with increased expression levels of bone morphogenesis protein (BMP)-2 in bone cells (1). The statin family reportedly increases bone mineral density in humans and decreases the risk of fractures in osteoporotic and elderly patients (2-4). Statins have not been clinically associated with a reduction in risk of fracture (5-6). Randomized controlled trials of the statin family are needed to confirm the risk of fractures (7).

The statin family enhances the mRNA and protein expression levels of BMP-2 and vascular endothelial growth factor (VEGF) to stimulate osteoblastic functions and upregulate gene expression of extracellular matrices including osteocalcin, bone sialoprotein, collagens and proteoglycans (2, 8-10). Statins induce and accelerate bone formation locally, trigger early development of growth factors, regulate angiogenesis by VEGF, differentiate into osteoblasts or osteocytes, and cause bone mineralization (11-12). Recently, Horiuchi and Maeda pointed out that statins may be useful for treating periodontal disease in patients with osteoporosis based on their review of the literature concerning statin and bone metabolism (13).

The proposed bone remodeling mechanism was involved in osteoblastic differentiation via BMP-2, which correlated with RhoA (13). Simvastatin is already employed in implant surgery to promote bone formation in vivo, and repeated local injection or an effective delivery method for local treatment is reportedly necessary (14).

Statins have also been involved in an immunomodulator that was reported to show a better prognosis for cardiac transplantation in patients under pravastatin treatment. Kwak et al. found that major histocompatibility complex class II (MHC-II) molecules are directly involved in activation of T cells and in the control of the immune response (15). Targeting of the statin binding site of leukocyte function antigen (LFA-1) could be used to treat diseases such as rheumatoid arthritis, psoriasis, ischemia and transplant rejection. LFA-1, integrin β2, has an important role in autoimmune diseases and nonspecific inflammatory
lesions (16). Simvastatin inhibits IL-17 secretion by inhibiting the expression of IL-17 transcription factor in CD4 lymphocytes (17). Positive effects of atorvastatin on LDL-C oxidation, platelet activation and inflammation involved in atherosclerotic processes are exerted after lowering LDL-C (18).

The present review deals with how the statin family effects bone regeneration in vitro and in vivo and also how it mediates new immunomodulators in MHC-II genes. Statins also selectively inhibit LFA-1 by binding to integrin. Additionally, the present paper notes rare but serious side effects, including myopathy and myositis, suggesting that statins may play a role in promoting muscle fiber damage (19).

1. Pharmaceutical mechanism of statins

Statins act at a rate-limiting step in cholesterol synthesis by blocking the conversion of HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) to mevalonate. Among statins, simvastatin and atorvastatin are very effective cholesterol-lowering agents that are widely used to reduce morbidity in coronary diseases. Statins are classified either as hydrophobic statins, including simvastatin, atorvastatin, and cerivastatin, or as the hydrophilic statin pravastatin (2). Compactin and mevastatin (ML-236B) were originally isolated as inhibitors of cholesterol synthesis from fungal metabolites, such as Penicillin Citrium and P. brevicompactum, and chemically synthesized or microbially converted new statins, simvastatin, pravastatin and others (20) (Table 1).

Statins are the prodrug of potent competitive inhibitors of HMG-CoA reductase in the liver microsomal enzyme, and include simvastatin. Lovastatin and cerivastatin show highly effective cholesterol-lowering action in humans and animals, and are useful in the treatment of coronary artery diseases. Lovastatin effects are blocked by mevalonate and less effectively by geranyl-geraniol, while alendronate effects are blocked partially by mevalonate and more effectively by geranylgeraniol (21). A mechanism regulates biosynthesis of mevalonate, the precursor of isoprenoid groups that are incorporated into many end products, including such sterols as cholesterol in membrane structure, Haem A and ubiquinone as electron transport, dolichol required for glycoprotein synthesis, isopentyladenine in some transfer RNAs and intercellular messenger farnesylated mating factors in fungi, juvenile hormones in insects, and steroid hormones in animals (22). The mevalonate pathway has already been exploited in cholesterol-lowering drugs. Inhibition of HMG-CoA reductase by lovastatin and related compounds blocks cholesterol synthesis and increases transcription of the LDL receptor gene.

2. Actions of statins

Among the known biological functions of statins are their pleiotropic effects other than lowering cholesterol, which include stimulation of nitric oxide (NO) production, anti-apoptosis (23-24), anti-proliferation (25) and immunomodulation (15). Statins are generally acknowledged to inhibit synthesis of cholesterol by inhibiting the rate-limiting enzyme, HMG-CoA reductase, and they prevent the synthesis of isoprenoid intermediates including farnesyl pyrophosphate (FPP) and geranylgeranyl phosphate (GGPP) (22). The system is essential for post-translational isoprenylation, and the activation of small GTP-binding proteins and a decrease in the isoprenylation may cause changes in cell function regulated by such proteins. Rho GTPase and downstream effector Rho-associated kinase reportedly play important roles in many cellular functions (26-27). Isoprenoids are lipids attached by post-translational modification to some small G proteins including Ras and Ras-like proteins (Rho, Rap, Rab and Ral). It is interesting to note that cerivastatin directly attenuates cardiac hypertrophy induced by endothelin in cultured rat myocytes partly by inhibition of the Rho pathway. JUPITER (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin), a survey study involving over 17,800 people in 26 countries, showed a significant reduction in the risk of cardiovascular disease with the use of one particular statin, rosuvastatin (28). Cerivastatin decreased endothelin-induced Rho protein expression, and mevalonate and geranylgeranyl phosphophate reversed this effect. These results suggest that cerivastatin directly attenuates cardiac hypertrophy by inhibition of the Rho pathway (29).

Bisphosphonate reportedly induces osteoclast apoptosis and also induces apoptosis in mouse J774 macrophages in vitro. It is likely that potent antiresorptive bisphosphonate

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<td>cerivastatin</td>
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also inhibits bone resorption by preventive protein prenylation in osteoblasts and that enzymes of the mevalonate pathway or prenyl protein transferases are molecular targets of nitrogen-containing bisphosphonates (30). The possible role of lipids has been implicated in regulating osteoblastic cells, although cholesterol is an important molecule that plays a key role in regulating cell function. Mevastatin inhibited the maturation of bone marrow stromal cells (MSCs) into functional osteoblastic cells. The cholesterol biosynthetic pathway is important for proper development of MSCs into functional osteoblastic cells capable of forming a mineralized matrix. This mechanism may provide new therapeutic approaches to prevent osteoporosis and bone aging (31).

Simvastatin enhanced phosphorylation of the endogenous Akt substrate endothelial nitric oxide synthase (eNOS), inhibited apoptosis and accelerated vascular formation in vivo in an Akt-dependent manner. In VEGF treatment, both simvastatin treatment and enhanced Akt signaling in the endothelium promoted angiogenesis in ischemic limbs of normocholesterolemic rabbits. Activation of Akt represents a mechanism that could account for some of the beneficial side effects of statins, including the promotion of new blood vessel growth (32). Fig. 1 summarizes major functions of statins.

### 3. Statins and mineralization

Simvastatin enhances mineralized nodule formation in culture, whereas coincubation with mevalonate, geranylgeranyl pyrophosphate, LY294002, or VEGF receptor 2 inhibitor (SU 1498) abrogated statin-induced mineralization. Statins stimulate VEGF expression in osteoblasts via reduced protein prenylation and the phosphatidylinositol-3 kinase pathway, promoting osteoblastic differentiation (2).

Statins regulate angiogenesis by VEGF, and pericytes differentiate into osteoprogenitor cells and osteoblasts (11-12, 33).

Experimental studies regarding bone formation and bone metabolism have employed simvastatin among several statin groups. Sugiyama et al. stated that simvastatin and compactin (mevastatin) activated BMP-2 promoter, whereas pravastatin did not (20). The statin-mediated activating BMP-2 promoter was completely inhibited by downstream metabolites of the HMG-CoA reductase, mevalonate, indicating that the activation was a result of the inhibition of the enzyme.

Osteoblasts arise from mesenchymal stem cell precursors and differentiation in response to a number of factors including BMPs, TGFβ, IGF-1, VEGF, hormones and glucocorticoids. In bone tissue, osteoblasts exist adjacent to endothelial cells, and VEGF inhibits bone formation and resorption. VEGF has mitogenic activity in the endothelial and smooth muscle cells as an angiogenic factor (34-35). Maeda et al. reported that simvastatin (10⁻⁶ M) time-dependently augmented VEGF mRNA expression in MC3T3-E1 cells, mouse stromal cells (ST2) and rat osteosarcoma cells (2). Transcriptional activation of a VEGF promoter-luciferase construct was significantly increased by simvastatin administration, and stimulation of the VEGF gene by simvastatin in MC3T3-E1 cells is transcriptional in nature. Statins stimulate VEGF expression in osteoblasts via reduced protein prenylation and the phosphatidylinositol-3 kinase pathway promoting osteoblastic differentiation and also mineralization.

The mechanism of simvastatin in induction of heat shock protein (HSP) has been examined in MC3T3-E1 cells. HSPs are classified into high-molecular weight HSPs (HSP70, HSP90 and HSP110) and low-molecular-weight HSPs (HSP27). Expression of HSP27 shows an increase
with the downregulation of proliferation in normal osteoblasts. Endothelin-1 stimulates HSP27 induction through activation of p38 mitogen-activated protein kinase in osteoblast-like MC3T3-E1 cells. Wang et al. indicated that simvastatin induces HSP27, HSP70 and HSP90 in MC3T3-E1 cells and simvastatin increased the levels of HSP27, but had little effect on the levels of HSP70 or HSP90 (36). These results strongly suggest that simvastatin does not stimulate the induction of HSP70 and HSP90, but indeed stimulates the induction of HSP27 in osteoblasts.

Song et al. found that simvastatin treatment enhanced the expression of mRNA of osteocalcin and protein of osteocalcin and osteopontin, increased alkaline phosphatase activity, and significantly inhibited adipocytic differentiation. Simvastatin induces high expression of BMP-2 in mouse bone marrow stromal cells, suggesting that it acts on these cells to enhance osteoblastic differentiation and inhibit adipocyte differentiation (37). Gimble et al. noted that rhBMP could inhibit adipocytic differentiation (38), but this inhibitory effect was not observed in the study of Song et al. (37). Simvastatin (10^{-7} M) markedly increased BMP-2 mRNA, VEGF, alkaline phosphatase, collagen type I, bone sialoprotein, and osteocalcin (OCN) in MC3T3-E1 cells, while suppressing gene expression of collagenase-1 and -3. In cultures of MC3T3-E1 cells, statins stimulate mineralization, and pretreating MC3T3-E1 cells with mevalonate or geranylgeranyl pyrophosphate (mevalonate metabolite) abolished statin-induced mineralization (9).

Effects of simvastatin treatment in a 1-year randomized trial on bone mineral density (BMD) have been reported in postmenopausal woman (39-40). Thirty consecutive postmenopausal hypercholesterolemic women were treated with simvastatin 40 mg/day for 12 months and 30 controls. The investigators suggested the probable positive effects of simvastatin on bone formation and BMD.

Lovastatin counteracts the effect of doxomethasone on the differentiation of bone marrow precursor cells into adipocytes (41-42). Lovastatin has also been associated with increased BMP-2 gene expression (1), alkaline phosphatase activity and matrix calcification. Li et al. reported that lovastatin acts on bone stromal precursor cells to modulate differentiation by enhancing osteoblast differentiation, through increased expression of the Cbfα1/Runtx2 gene and also by increasing activity of the osteocalcin promoter (43). Lovastatin inhibits adipocytic differentiation, apparently by acting on the expression of fat cell genes, PPARγ2 and 422aP2, and subsequent maturation. Lovastatin may stimulate shunting of uncommitted precursor cells from the adipocyte differentiation pathway into the osteoblast differentiation pathway (12).

The effects of simvastatin and atorvastatin have been demonstrated on osteoblastic activity by cell proliferation analysis, and on collagen, osteocalcin, and BMP-2 gene expression using hOB and MG-63 cell lines. Results show a statistically significant decrease in cell proliferation related to simvastatin or atorvastatin addition at all concentrations of primary hOB cells compared to those not treated. A significant increase in collagen, osteocalcin and BMP-2 gene expression was detected when hOB culture was treated with both statins at different concentrations. These findings support the bone-forming effect of statins, probably through the BMP-2 pathway (10).

4. Statins act on bone tissue

Among several functions of the statin family, the mechanism by which statins affect bone tissue and cell function is an important issue. Statins stimulate bone formation in vivo and in vitro and increase bone volume in cultured mouse calvaria. The enhancing effects of statins on bone formation are associated with increased expression of BMP-2 via activation of the gene promoter (1, 9). A relatively low dose of simvastatin (10^{-7} M) reportedly induced osteoblastic differentiation and markedly increased mineralization in MC3T3-E1 osteoblasts (44). Statins, compactin and pitavastatin promote differentiation of embryonic stem cells into osteoblasts, and up-regulate gene expression for BMP-2 and osteocalcin by depletion of mevalonate (45). Finally, BMP-2 plays a role in the osteoblastic maturation induced by statins. It is well known that several factors influencing bone metabolism, TGFβ, FGF2, IGF-I, and VEGF, are involved in promoting differentiation of osteoblasts and stimulating bone regeneration. Statins are known to rapidly activate the protein kinase Akt/ PKB in endothelial cells, and simvastatin enhances phosphorylation of the endogeneous Akt substrate endothelial nitric oxide synthase (eNOS), inhibits apoptosis and stimulates vascular formation in vitro in an Akt-dependent manner (45).

VEGF treatment with simvastatin administration and enhanced Akt signaling in the endothelial cell promotes angiogenesis in ischemic limbs (32). Involvement of VEGF in endochondral ossification has been demonstrated by VEGF inactivation, which restricts formation via inhibition of angiogenesis (46). Hydrophilic statins, simvastatin, atorvastatin and cerivastatin, but not a hydrophilic statin, pravastatin, markedly increase VEGF mRNA abundance in MC3T3-E1 cells (2). Vascular endothelial cells and osteoblasts produce VEGF and the expression is enhanced by factors, including prostaglandin E1, IGF-1, TGF-β, FGF2 and D3. Osteoblasts express the VEGF receptor, VEGF, P-2 (Hk-1), which binds VEGF-A, VEGF-C and VEGF-D isoforms with high affinity (47). VEGF is a much more potent inducer of osteoblastic differentiation than BMP-2 (33). Statin augments mRNA expression for VEGF by these osteoblastic cell lines, indicating that statin induces the expression.

Humans generally show bone loss with aging, and the effects of treatments by cerivastatin and parathyroid hormone (PTH) have also been reported in aged rats. It was concluded that cerivastatin does not prevent age-related bone loss, whereas PTH prevents it in the vertebra (48).

5. Clinical use of statins

At present, in vivo studies have shown that statins are a
promising candidate for bone formation compounds, and local administration of statins promotes new bone formation by histological and X-ray examination of extracted tooth sockets, peri-implanted tissue regeneration, residual alveolar ridge and statin-containing collagen grafts. Wong and Rabie reported that statin-collagen grafts are osteoinductive bone grafting materials that can be used alone in small bone defects or in combination with autogenous bone grafts (11).

Simvastatin accelerated osteogenesis locally and triggered early expression of VEGF, BMP and Cbfal in angiogenesis and then differentiation into bone cells (osteogenesis) in rabbits (49). Remodeling of alveolar bone following tooth extraction in Wistar rats has been obtained with radiographic and histopathologic examinations. Simvastatin was used with poly lactide-co-glycoside (PLGA) as the carrier, and experimental rats were killed after implantation. The results indicate that local application of simvastatin could effectively preserve residual alveolar bone by promoting bone formation in the extracted socket (50). Subcutaneous administration of simvastatin stimulates ectopic ossification by rhBMP-2 through the reduction of bone turnover. Simvastatin may become a useful adjuvant for BMP-2 induced bone formation, and it is useful for repair of bone defects of cleft palate and for reconstruction following surgical resection (51).

Rejnmark et al. treated 82 postmenopausal women with simvastatin 40 mg/day or a placebo for 1 year (40). The results showed no effect of simvastatin on biochemical bone markers or on BMP at the hip or spine. However, a significant increase in BMP was found in response to simvastatin at the forearm.

Recently, Maritz et al. examined the effects of different doses of simvastatin (1, 5, 10, 20 mg), atorvastatin (2.5 mg) and pravastatin (10 mg/kg/day) orally administered for 12 weeks on femoral BMD and quantitative bone histomorphometry (QBH) in rats with or without ovarioectomy. The results indicated that statins decrease BMD, and high-dose simvastatin increases bone formation and resorption; however low-dose simvastatin decreases bone formation and increases bone resorption. The effect of simvastatin on QBH differs with different dosages, and that seen in intact rats and not in ovarioctomized rats. Simvastatin is unable to prevent bone loss caused by ovarioectomy. Differences in statin dosages, duration, sex of the experimental animals, and methods of administration to the animals may explain the contrasting results as positive or negative effects (52).

In order to clarify the mechanism of statin effects for prevention of bone loss and fractures, Viereck et al. evaluated the effects of atorvastatin on osteoblastic production of receptor activator nuclear factor-κB ligand (RANKL), osteoprotegerin (OPG) and cytokines. In primary human osteoblasts (hOB), atorvastatin increased OPG mRNA levels and protein secretion by hOB up to three-fold in a dose-dependent manner with a maximum effect at 10^{-6} M. The data suggest that atorvastatin enhances osteoblastic differentiation and production of OPG (53). Most statins, including atorvastatin, are lipophilic compounds which undergo a substantial first-pass effect in the liver after oral administration, and their affinity to bone is low. In contrast, bisphosphonates, which by virtue of chemical structure are preferentially bound to hydroxyapatite within the bone tissue, result in high local concentrations. Lipophilic statins appear to have a more potent bone-sparing effect as compared to hydrophilic statin pravastatin. Since osteoblastic OPG production is positively correlated with the stage of differentiation (54), the stimulatory effects of atorvastatin on osteoblastic OPG production may be related to its capacity to enhance osteoblastic differentiation. BMP-2 is reported to be an important stimulator of OPG mRNA levels and protein secretion in human osteoblastic cells (55). Enhancement of osteoblastic BMP-2 expression and differentiation by statins is directly related to inhibition of Rho-associated kinase, which depends upon the availability of prenylated intermediates. OPG production is a function of osteoblastic cell maturation, and enhancement of OPG by atorvastatin may be related to the stimulatory effects on osteoblastic differentiation.

Hofbauer and Schoppet underscored the clinical implications of the OPG/RANKL/RANK system for bone and vascular diseases (56). Their study reviewed a bone remodeling system by bone formation with osteoblasts and bone resorption with osteoclasts, a balanced process which continuously remodels in bone tissue. RANKL is identified as an essential cytokine for the production and activation of osteoclasts. Biological effects of RANKL are physiologically counterbalanced by the decoy receptor OPG. It is known that reduced estrogen synthesis, glucocorticoid-treated rheumatoid arthritis (T-cell activation), and bone tumors (metastases, myeloma) enhance the ratio of RANKL to OPG, promote osteoclastogenesis, then accelerate bone resorption and finally cause bone loss. Metabolic changes of the OPG/RANKL/RANK system have been implicated in vascular diseases. RANKL blockade has prevented bone loss caused by osteoporosis and may emerge as the therapy of choice for postmenopausal osteoporosis, myeloma and osteolytic tumor metastasis in humans.

Randomized trials of statin administration to reduce fracture risk have been reported (57). Among 91052 patients in the final cohort, 28063 were prescribed statins and 2195 were given non-statin lipid-lowering medications. In adjusted analysis, statin use was associated with a 36% (odds ratio 0.64, 95% confidence interval 0.58–0.72) reduction in fracture risk when compared with no lipid-lowering therapy and a 32% (odds ratio 0.67, 95% confidence interval 0.50–0.91) reduction when non-statin lipid-lowering therapy was used. The study was conducted by the New England Veterans Affairs Health Care System between January 1998 and June 2001 in a large population of elderly, predominantly male veterans. Statin therapy reportedly has had serious and rare side reactions including myositis and myopathy as a feature of pathological breakdown of skeletal muscle and acute renal failure. In a large population study involving more than 250,000 patients using several statins, the incidence of myolysis was 0.44 per 10,000 patients and the risk of cerivastatin was more than ten-fold greater. Thus in 2001 the use of cerivastatin was discontinued (58). Another
study noted that the risk of myopathy is lowest with pravastatin and that lovastatin induces the gene atrogin-1, which is responsible for enhanced damage of skeletal muscle (59).

**Conclusion**

Based on the findings from clinical trials, as well as the National Cholesterol Education Program guidelines, there has been an increased focus on LDL-cholesterol lowering statins and their important roles in the prevention of coronary heart disease, myocardial infarction, stroke and peripheral arterial lesions. LDL-lowering potency varies among the statin family members, and the effects of statins on bone have not yet been fully established. Statin therapy rarely causes and serious reactions such as muscle damage, myositis and myopathy. The role of statins in bone formation and in cholesterol metabolism must be explored more extensively.

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